

The formulation, analysis and stability of glyceryl trinitrate in non-aqueous pharmaceutical formulations

By Brian K. Evans, PhD, FRPharmS, Kay Potbecary, BSc, Lynne Hutchings BSc, and John Rhodes, MD

AIM • To develop formulations (capsule, ointment, suppository) of glyceryl trinitrate for the topical treatment of ileal, colonic and anal fissures. To analyse these formulations and assess their stability.

METHOD • The three lipophilic formulations of glyceryl trinitrate were manufactured and packaged according to Good Manufacturing Practice. Extraction of GTN from each preparation was based on the method in the United States Pharmacopoeia/ National Formulary monograph for nitroglycerin ointment. Two comparative extraction methods were also developed. Uniformity of drug distribution in each dosage form and its long-term stability were determined using high pressure liquid chromatography (HPLC) analysis.

RESULTS • Consistent manufacturing procedures were established for the three lipophilic formulations. Methanolic extraction of GTN from each of these products was more efficient than deionised water alone. The analytical consistency achieved with methanolic extraction confirmed this as the preferred method of analysis.

CONCLUSIONS • Validated manufacturing and analytical processes were established for the three formulations. GTN can be extracted efficiently from non-aqueous bases and analysed by HPLC. Intra-batch sampling provided a means of confirming uniform drug distribution in each manufactured formulation. Product expiry dates were also established using the stability data for the GTN content in each preparation.

Glyceryl trinitrate (GTN) and other organic nitrates have been used for many years as vasodilators in the treatment of cardiovascular disease. Taken sublingually they provide rapid relief for angina pectoris, and may be given orally or transdermally for maintenance therapy. Intravenous preparations are also used for unstable angina and severe left ventricular failure. Glyceryl trinitrate is available as a range of oral and intravenous presentations to treat these conditions.

Following administration, organic nitrates are taken up by the vascular endothelium and degraded by cellular metabolism to release nitric oxide,¹ which in turn produces vasodilation. Organic nitrates are prodrugs for nitric oxide, an unstable compound with a short half-life of about a second, which is rapidly oxidised to nitrate and nitrite.

Chronic anal fissures have traditionally been treated by surgical division of the internal anal sphincter to reduce anal canal pressure, which in turn promotes healing. The discovery of nitric oxide as a major inhibitory neurotransmitter involved in relaxation of the sphincter presents a possible alternative treatment and recent clinical studies with topical application of suitable GTN preparations have been shown to be effective.

In the smooth muscle of the gut, nitric oxide plays an important role as an inhibitory non-adrenergic, non-cholinergic neurotransmitter.² In the gut it produces muscle relaxation. The release of nitric oxide from GTN reduces anal pressure and promotes healing in the chronic anal fissures of patients³ by increasing perianal skin blood flow. Both GTN suppositories and ointments have been used to treat anal fissures.

Parallels can be drawn between these uses of nitrates and their potential role in inflammatory bowel disease in which mucosal fissures and ischaemia are features. In theory local mucosal bioavailability of GTN (acting as a nitric oxide donor) would produce vasodilation and smooth muscle relaxation, increasing blood flow in the tissues and thereby promoting fissure healing. A delayed and sustained release oral formulation would be ideal, providing uniform release of the GTN in the distal small bowel and colon.

To treat these conditions it would be necessary to design and validate three manufacturing methods, to select preferred extraction methods, to determine intra-batch variation for each formulation and to assess long-term product stability.

METHOD

Formulations The suppositories, ointments and capsules were formulated to provide a constant release rate of GTN when administered rectally, applied topically to the rectal mucosa, or taken orally. GTN suppositories and ointments were prepared to treat anal fissures, and the capsules to treat inflammatory bowel disease.

The suppository A hard fat suppository base, Witepsol S55 (Huls AG), was selected for

the rectal delivery of GTN. This lipophilic base consists of mixtures of triglyceride esters of the higher saturated fatty acids (C₉H₁₉COOH to C₁₇H₃₅COOH) and varying proportions of mono- and diglycerides. It has a melting point range of 33.5°C to 35.5°C.

The suppository base was heated to 40°C and the GTN powder incorporated into this melt using a Silverson L4R mixer fitted with a dispersion attachment. The suspension was then poured into the hopper of a Hibar Liquid Filling Capsule Machine (Shianogi), maintained at 40°C and stirred continuously. This was dispensed into 1g polypropylene suppository moulds (Homeo-Technik). This was achieved by using an in-house modification of the filling machines moving carriage which holds the moulds and is situated below the delivery pumps nozzle.

The GTN content of the suppositories were 250µg, 500µg and 1mg.

The ointment A soft, occlusive ointment base was prepared by warming equal parts of hydrous wool fat ointment and yellow soft paraffin in a heated mixing bowl (Crypto Peerless EC20 mixer fitted with a suitable paddle for viscous formulations). The GTN powder was then incorporated into this melt. The ointment was packed into 30g white lacquered aluminium ointment tubes (Adelphi Mfg). A strength of 0.2%w/w was prepared.

The capsule The GTN powder was incorporated in a melted mixture of polyglycolised triglycerides (Gelucires, Gattefosse) held at 55°C and stirred using the same Silverson mixer and fitting as described earlier. The composition of the matrix was Gelucire 42/12 17.5%w/w, Gelucire 44/14 7.5%w/w,

Dr Evans is clinical research pharmacist, Ms Potbecary is pharmacy technician and Ms Hutchings is senior pharmacy technician at SMPU, The Quadrant Centre, Cardiff Business Park, Llanishen, Cardiff CF14 5RA. Dr Rhodes is emeritus professor of gastroenterology at the University Hospital of Wales, Cardiff. Correspondence to Dr Evans

and Gelucire 50/13 75%w/w. This provided a sustained release of the drug over six hours.

The in-house manufacture of the size 1 hard gelatin capsules (Capsugel) was carried out using the Hibar Liquid Filling Machine (as described earlier) with an additional capsule filler fitting attached to the moving carriage. Later commercial manufacture used a Schweizer mixer and a Bosch 1,500L filling machine.

The capsules were coated with an acrylic resin (Eudragit L) which has an optimum dissolution rate at pH6.8 and above. In-house coating used a Labotec capsule coating machine and subsequent commercial coating used a Manesty Accelacota 10. Each capsule contained 3mg of GTN.⁴

The drug Glyceryl trinitrate is only commercially available diluted with either lactose, dextrose, alcohol or propylene glycol. A 2%w/w GTN in lactose powder was preferred and was readily available (Dipharma Italy). Although accompanied by full documentation including a Certificate of Analysis, safety information and long-term stability data, routine in-house analysis of this powder was carried out.⁵

Analysis Analytical procedures and techniques were developed using as guidance those appearing in the 1995 United States Pharmacopoeia/National Formulary (USP 23/NF18)⁶ monograph for nitroglycerin ointment. Some sections of this monograph appear for the first time in the British Pharmacopoeia 2001 monograph for glyceryl trinitrate tablets.⁷ However this entry is principally for related substances only, and not as an assay procedure for total GTN content.

High-pressure liquid chromatography A degassed eluent solution containing equal volumes of methanol and deionised water was prepared. The chromatographic system uses an ultraviolet diode array detector at 210nm, a Spherisorb 5ODS 2 column (25cm x 4.6mm internal diameter), a guard column and a flow rate of 1ml/min. The injection volume was 25µl. Sample injection was performed using a Hewlett Packard (HP) 1100 series auto-sampler, with an HP1000 series quaternary pump, and peak areas were recorded using a Hewlett Packard 3392A integrator. The computerised integration/control system was a Hewlett Packard Chem Station-Windows Application.

A slightly acidic (pH=5.5) methanolic stock solution of pure GTN was prepared by dissolving 1g of the glyceryl trinitrate 2%w/w in lactose powder in 50:50 methanol:deionised water and made up to 50ml. This gave a GTN concentration of 0.04%w/v (= 400µg/ml). A series of known volumes of this were then diluted in the methanol:deionised water mixture to give calibration solutions of concentrations 0.0032%w/v (32µg/ml), 0.0036%w/v (36µg/ml), 0.004%w/v (40µg/ml), 0.0044%w/v (44µg/ml), and 0.0048%w/v (48µg/ml).

Using 25µl aliquots these were analysed in duplicate, integrated and stored as the calibration curve. A correlation coefficient greater than 0.99 was acceptable.

Although a British Pharmacopoeial standard (BPCRS) for GTN was available as a nominal 1%w/v solution in 96 per cent ethanol (with a declared content of 98.6 per cent), this offered no advantage over the use of the powder formulation for this calibration exercise.

Extraction of the GTN As already mentioned our modified extraction procedure was based on the 1995 USP 23/NF18 monograph for nitroglycerin ointment. Initially random batch samples were taken from completed batches of the ointment, suppository and capsule. These were accurately weighed to give an equivalent of 1mg of GTN in 25ml of extraction solvent. In the first series of extractions, deionised water and methanol were used as separate solvents. In the case of the capsule and suppository these were firstly sliced and then all three samples placed in a 50ml volumetric flask, adjusted to pH 5 to 5.5, and made up to volume. The flasks were placed in a thermostatically controlled water-bath at 55C for four hours and shaken intermittently. The flasks remained in the water-bath, allowed to cool overnight, and then adjusted to volume.

The content of each flask was centrifuged at 4,000rpm for five minutes, sufficient of the supernatant drawn up in a syringe and distributed into HPLC vials via a Whatman 13mm GD/X disposable, 45micron syringe filter.

The second and third series of extractions followed this procedure but only used methanol as solvent.

RESULTS

The HPLC calibration standards used for the three extractions gave linear calibration curves ($r=0.999$, 0.998 , and 0.994 , respectively) which passed through the origin of the graph (Figure 1). The GTN retention time was 8.9mins, and its characteristic peak is shown in Figure 2.

Comparison of the results for the two initial methods of extraction showed good correlations for the capsule and ointment formulations (range 91.2–108.2%w/w) in both media but only for the methanolic extraction in the case of the suppository (106.4%w/w). These results are shown in Table 1. Visual appearance of the extraction flasks showed greater clarity of the supernatant methanolic solution when compared with the aqueous solution. Consequently only methanol was used in subsequent extractions.

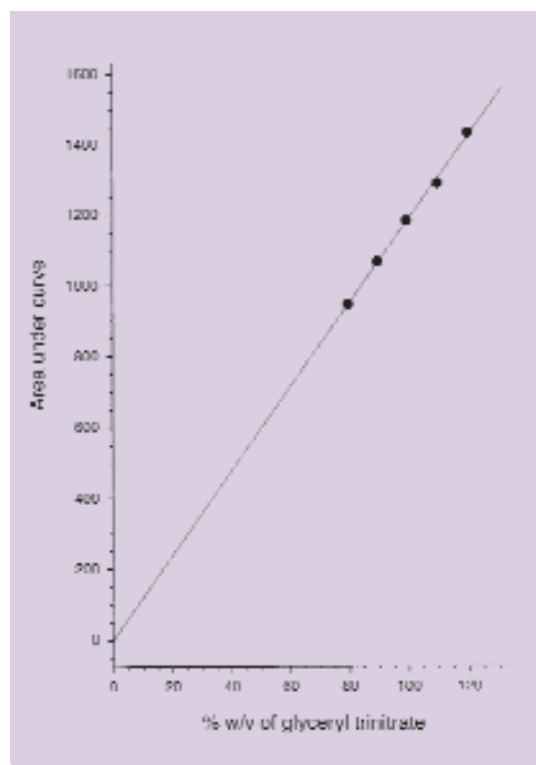


Figure 1: Calibration for glyceryl trinitrate

The second series of extractions examined intra-batch variation. Six random samples were taken from different levels in the manufacturing vessel used for batches of capsules, ointment and suppositories. Average GTN contents of the stated dose for each dosage form were 102.1%w/w, 80.5%w/w, and 73.7%w/w, respectively (Table 2). With the exception of the capsules, the average figures were lower and the ranges wider than expected.

In the third series, six batch-retention samples of the capsules, ointments and suppositories were analysed. Average drug concentrations were 101.5%w/w, 103.3%w/w, and 73.7%w/w, respectively (Table 3). The capsule samples were taken from five batches manufactured in-house and one batch manufactured commercially. The ointment and suppository samples were from batches manufactured over a six-month period. These results indicated good product stability over the period for the capsule and ointment formulations but were less favourable for the suppositories.

The pH values of the capsule matrix, the ointment and suppository bases were 4.2, 6.2, and 3.8, respectively.

DISCUSSION

GTN is slightly soluble in water (1 in 800) and freely soluble in alcohol (1 in 4). It is stable in weakly acidic and neutral solutions, but is rapidly hydrolysed in strongly alkaline solutions to form the alcohol and nitrate ion.⁵ Although the stability of GTN in hydrophilic formulations is well documented there is little published data on the drugs stability in non-aqueous (lipophilic) formulations.

GTN is now routinely used to treat anal fissures. This condition, together with its possible use in treating Crohn's disease, has

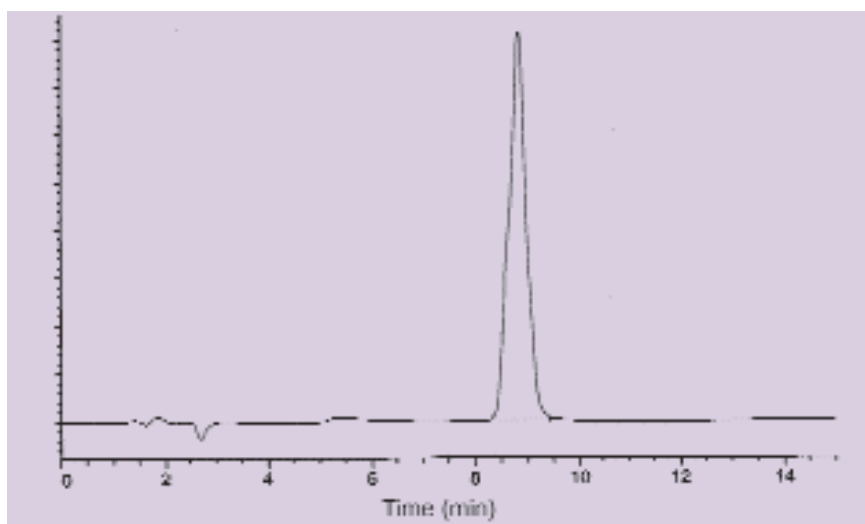


Figure 2: Chromatogram of glyceryl trinitrate

GTN formulation	Recovery of stated dose	
	Aqueous extraction	Alcoholic extraction
Capsules 3mg	99.6	103.4
Suppositories 1mg	26.5	106.4
Ointment 0.2%w/w	91.2	108.2

Results are the average of duplicate injections

GTN formulation	% stated dose	Range (%)
Capsules 3mg	*102.1	92.1–105.7
Suppositories 1mg	*73.7	65.5–82.0
Ointment 0.2%w/w	*80.5	78.0–109.0

*n=6

GTN formulation	% stated dose	Range (%)
Capsules 3mg	*101.5	94.5–103.6
Suppositories 1mg	*73.7	67.6–92.0
Ointment 0.2%w/w	*103.3	70.2–104.0

*n=6

highlighted the need for suitable non-aqueous formulations to be developed. In pharmaceutical terms these bases should provide a stable environment for the drug. However their hydrophobicity may affect the drug's availability when the preparation comes in contact with the gastrointestinal or rectal mucosa. It may also affect the extraction process due to tight GTN adsorption on to one or more of the lipophilic components of the formulations.

The formulation's components could also affect *in vivo* GTN bioavailability by providing a slower release of GTN, which may well prove beneficial not only by prolonging a local therapeutic effect but also by minimising systemic absorption where high levels of the drug are responsible for causing headaches, its principal side effect. In some sensitive patients this problem tends to restrict treatment.

Effective product application is important and in the case of the suppository base a

surfactant has been added to improve dispersion of the base *in situ* and thus the availability of the incorporated GTN. Similarly the ointment's consistency was such as to allow uniform smearing on to the anal mucosa. The polyglycoside triglycerides used in the capsule formulation are physicochemically similar to the suppository base but these range of products are identified by their individual melting points and hydrophilic/lipophilic (HLB) numbers. These physical characteristics were important factors to consider in conjunction with those of the drug during product development. A formulation was eventually designed that met our criteria in terms of its melting point, drug release pattern and bioavailability. These properties would come into play following disintegration of the capsule coating in the proximal small bowel releasing the wax matrix containing the GTN.

There is now strong clinical evidence which supports the ointment as the preferred formulation to treat anal fissures, a fact that is now reflected in our production figures. The extraction values for the ointment were consistently better than the suppositories throughout the analyses. These figures also showed that the methanol extraction was more effective than the deionised water extraction.

Inefficient extraction, uneven distribution of GTN in the ointment and suppository bases during manufacture, or the unstable nature of GTN may explain some of these poor results. However following modification of the manufacturing processes the analytical results have been good. It is unlikely that the earlier results are related to product stability since the values do not correlate with the period of storage. The analytical results for the capsules were the most consistent throughout.

Recognising the difficulties encountered when analysing GTN formulations official US and British Pharmacopoeias have set wider content limits (90–115 per cent and 85–115 per cent, respectively) for these products. This was one of the reasons why we initially looked at an alternative aqueous extraction method, which, although successful for the capsules and

ointments, failed to improve these figures for the suppositories.

Increasing the water-bath temperature by five degrees (from 50°C) and maintaining it for four hours (compared with 10 minutes in the official USP/NF monograph) allowed additional time for the drug to dissociate from the lipophilic components of the preparations.

As well as establishing a routine analytical procedure for quantitative GTN measurements in non-aqueous bases, this work has provided a validated method for determining long-term stability of the three formulations. The HPLC method for GTN has been shown to be reliable and reproducible and is now in routine use in this laboratory. It is now also used for analyses of a range of similar isosorbide dinitrate formulations.

CONCLUSION

Manufacturing processes and procedures for the suppositories and ointment were refined in the light of the analytical results. The introduction of longer mixing periods before packaging resolved the identified problem of intra-batch variation. There is now an established manufacturing process and validated analytical procedure for each product.

ACKNOWLEDGEMENTS Our thanks to Colin Ranshaw and Dr Sarah Hiom, at the SMPU, and David Llewellyn at Media Resources, for their help, constructive comments and criticisms of this work.

This paper was accepted for publication on 20 February 2003.

REFERENCES

- Palmer RMH, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelial relaxation factor. *Nature* 1987;327:524–6.
- Brookes SJH. Neuronal nitric oxide in the gut. *J Gastroenterol Hepatol* 1993;8:590–3.
- Lund JN, Scolfield JH. A randomized prospective double-blind placebo-controlled trial of glyceryl trinitrate ointment in the treatment of anal fissure. *Lancet* 1997;349:11–14.
- Hawkes ND, Richardson C, Chng CL, Green JT, Evans BK. Enteric-release glyceryl trinitrate in active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Aliment Pharmacol Ther* 2001;15:1867–73.
- Technical Documentation: Nitroglycerin 1998. Dynamite Dipharm SpA Prov di Udine, Italy
- US Pharmacopoeia/National Formulary 23/18:1091–2. Washington: USP Convention Inc; 1995.
- British Pharmacopoeia 2001. Volume 2. London: The Stationery Office; 2001. pp2125–6.